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Xiao, Ning ; Qiu, Jiamin ; Nakao, Minoru ; Nakaya,
Kazuhiro ; Yamasaki, Hiroshi ; Sako, Yasuhito ; Mamuti,
Wulamu ; Schantz, Peter M. ; Craig, Philip S. ; Ito, Akira

SHORT REPORT: IDENTIFICATION OF *ECHINOCOCCUS* SPECIES FROM A YAK IN THE QINGHAI-TIBET PLATEAU REGION OF CHINA

NING XIAO, JIAMIN QIU, MINORU NAKAO, KAZUHIRO NAKAYA, HIROSHI YAMASAKI, YASUHIITO SAKO, WULAMU MAMUTI, PETER M. SCHANTZ, PHILIP S. CRAIG, AND AKIRA ITO

Department of Parasitology and Animal Laboratory for Medical Research, Asahikawa Medical College, Asahikawa, Japan; Sichuan Institute of Parasitic Diseases, Chengdu, Sichuan, China; Division of Parasitic Diseases, National Centers for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; Bioscience Research Institute, School of Environment and Life Sciences, University of Salford, Great Manchester, United Kingdom

Abstract. The species identification of an echinococcal lesion in the liver of a yak in the Qinghai-Tibet plateau region of China, where both *Echinococcus granulosus* and *E. multilocularis* are present, was difficult to determine because of the atypical appearance of the lesion. Polymerase chain reaction-based mitochondrial genotyping allowed us to discriminate the *Echinococcus* species. Nucleotide sequences of the cytochrome *c* oxidase subunit 1 (*cox1*) and cytochrome *b* (*cytb*) genes amplified from the echinococcal lesion demonstrated that the yak was infected with the *E. granulosus* G1 genotype (sheep strain).

The canine intestinal tapeworms *Echinococcus granulosus* and *E. multilocularis* are important zoonotic pathogens that cause serious disease in humans.^{1,2} In general, *E. granulosus* produces unilocular cysts in liver and lungs of ungulates and humans, whereas *E. multilocularis* causes alveolar cystic lesions in rodents and humans. The liver of susceptible intermediate hosts is the most common site of development for both parasites and the causative species is often misidentified when the metacestode is poorly developed in unsuitable intermediate hosts.

The yak *Bos grunniens*, a bovid species, inhabits steppes of the Himalayan highlands and was domesticated in Tibet about 3,000 years ago.³ The native people are totally dependent on their yak herd to support their livelihood. Although public slaughter houses are available in Tibet, domesticated yaks are frequently killed in individual households. Their viscera discarded carelessly become baits for dogs and foxes. Moreover, these canines scavenge on dead yaks in winter.^{4,5} Thus, living with canines in grasslands is a risk factor of echinococcosis for Tibetan livestock farmers.⁶

Shiqu County, located in Qinghai-Tibet plateau region of western Sichuan in China, is a highly endemic area for both cystic and alveolar echinococcosis.⁷ Based on gross and microscopic appearance, liver lesions in 6 (6.2%) of 97 yaks from Shiqu County were tentatively identified as *E. multilocularis*.⁵ The presence of *E. multilocularis* in humans and other hosts from the same region have supported this assumption.^{4,7} In another survey including a neighboring region (Ganzi County), echinococcal lesions were detected in 221 (51.5%) of 429 yaks.⁴ These were morphologically classified as *E. granulosus*-like polycystic types (50.8%, 218 of 429) and *E. multilocularis*-like alveolar types (0.7%, 3 of 429). Therefore, we attempted to confirm identification using molecular techniques that discriminate between *E. granulosus* and *E. multilocularis* infections.

A presumptive echinococcal liver lesion from a domesticated yak in Shiqu County was transported to our laboratory for molecular typing of the *Echinococcus* species. The lesion was characterized by small alveolar vesicles and necrotic areas (Figure 1A). An intense fibrogenesis was observed around the vesicles in which germinal layers were poorly developed (Figure 1B). These features were similar to those of human lesions caused by *E. multilocularis*. The occurrence of aberrantly developed *E. granulosus* lesions in cattle was de-

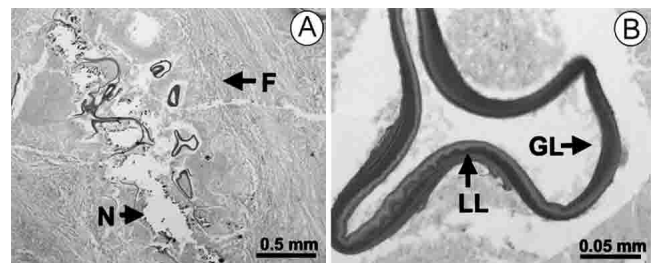


FIGURE 1. Echinococcal liver lesion in a yak (periodic acid-Schiff stain). **A**, low-power magnification. **F** = fibrogenesis; **N** = necrotic area. **B**, high-power magnification. **GL** = germinal layer; **LL** = laminated layer.

scribed earlier by Vogel, who pointed out their superficial similarity to the metacestode of *E. multilocularis*.⁸ He suggested that these lesions should be referred to as “multicystic” or “multivesicular” rather than “multilocular”.

Genomic DNA was extracted from the lesion by using the DNeasy tissue kit (Qiagen, Hilden, Germany) and then used as a template for a polymerase chain reaction (PCR). Mitochondrial genes of *Echinococcus* species were amplified by using the following primer pairs: primers F/CO1 (5'-TTGAATTTGCCACGTTTGAATGC-3') and R/CO1 (5'-GAACCTAACGACATAACATAATGA-3') for cytochrome *c* oxidase subunit 1 (*cox1*) gene, and primers F/Cytb (5'-GTCAGATGTCTTATTGGGCTGC-3') and R/Cytb (5'-TCTGGGTGACACCCACCTAAATA-3') for cytochrome *b* (*cytb*) gene. The PCR was carried out in a 50- μ L reaction mixture containing 2 μ L of template DNA, 200 μ M of each dNTP, 0.2 μ M of each primer, 1 unit of *Taq* polymerase (Ex *Taq*; TaKaRa Biomedicals, Tokyo, Japan), and Ex *Taq* reaction buffer. For PCR amplification, we used 35 thermal cycles (94°C for 30 seconds, 54°C for 30 seconds, and 72°C for one minute). The PCR amplicons were directly sequenced by using a dye terminator cycle sequencing kit (DYEnamic ET Terminator; Amersham Biosciences, Tokyo, Japan) and an ABI PRISM 377 sequencer (Perkin Elmer Applied Biosystems, Foster City, CA).

As shown in Table 1, partial sequences of *cox1* (795 basepairs) and *cytb* (568 basepairs) genes obtained from the yak lesion were compared with previously reported sequences of *Echinococcus* species.^{9,10} Both sequences were identical with

TABLE 1

Mitochondrial genes of *Echinococcus* species amplified from a liver lesion of a yak in the Qinghai-Tibet plateau region of China

Gene (accession no.)	Length sequenced (basepairs)	% similarity* compared with	
		<i>E. granulosus</i> G1	<i>E. multilocularis</i>
<i>Cox1</i> (AY278068)	795	99	90
<i>Cytb</i> (AY278067)	568	99	85

* Values were calculated with the Clustal W program (<http://clustalw.genome.ad.jp>).

those of the *E. granulosus* G1 genotype (sheep strain); however, a transitional change (C/T) at position 532 occurred in *cox1* gene and a transversional change (C/A) at position 140 occurred in the *cytb* gene. These data confirm that the yak was infected with the sheep strain of *E. granulosus*, rather than *E. multilocularis*. The result is consistent with the observation that yaks are kept with sheep in Shiqu County.

Previous molecular studies have revealed a number of diverse genetic strains of *E. granulosus*.^{11,12} To date, eight distinct genotypes (G1–G8) have been identified.¹³ Among them, G1 and G6 (camel strain) genotypes have been previously recorded in China.¹⁴ The fertile cysts of yaks were identified as the *E. granulosus* G1 genotype,¹⁵ suggesting that yaks significantly contribute to the transmission of cystic echinococcosis in China. However, as shown in this study, it is suggested that the yak is not an adequate host, since metacystode development in the yak is arrested. The identification of echinococcal lesions in yaks as *E. granulosus* rather than *E. multilocularis* should be confirmed by molecular typing of a larger number of samples because domesticated yaks are grazed widely in areas where *E. multilocularis* is known to occur in small mammals and canines. The precise understanding of *Echinococcus* species in domestic animals is important for control of echinococcosis in China.

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Authors' addresses: Ning Xiao, Minoru Nakao, Kazuhiro Nakaya, Hiroshi Yamasaki, Yasuhito Sako, Wulamu Mamuti, and Akira Ito, Department of Parasitology and Animal Laboratory for Medical Research, Asahikawa Medical College, Midorigaoka-Higashi 2-1, Asahikawa, 078-8510, Japan, Telephone: 81-166-68-2420, Fax: 81-166-68-2429, E-mail: akiraito@asahikawa-med.ac.jp. Jiamin Qiu, Sichuan Institute of Parasitic Diseases, Chengdu 610041, Sichuan, China. Peter

M. Schantz, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 4770 Buford Highway, Atlanta, GA 30341. Philip S. Craig, Bioscience Research Institute, School of Environment and Life Sciences, University of Salford, Great Manchester, M5 4WT, United Kingdom.

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